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**AUTOLOGOUS PERFUSION TECHNIQUE
FOR AN ISOLATED GASTROINTESTINAL
TRACT OF A RABBIT**

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By

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MODELING OF THE NON-AUDITORY RESPONSE TO BLAST OVERPRESSURE

Autologous Perfusion Technique for an Isolated Gastrointestinal Tract of a Rabbit

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AUTOLOGOUS PERFUSION TECHNIQUE FOR AN ISOLATED GASTROINTESTINAL TRACT OF A RABBIT

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ABSTRACT

Most perfusion techniques rely on mechanical means to provide blood flow to the isolated organ for maintaining its physiological conditions. The approach usually requires a complicated mechanical system with the associated problems of blood type matching and prevention of blood cell damage. This paper describes a gastrointestinal tract perfusion technique developed to use the rabbit's own cardiopulmonary system as the autologous blood supply source. The technique involves the removal of the complete intestinal loop, duodenum to descending colon, from the abdominal cavity of the rabbit, and maintaining its blood circulation through silastic tubing connections of the catheterized portal vein and cranial and caudal mesenteric arteries. The isolated perfused GI tract may then be placed in a separate test environment for controlled experiments. For a terminal animal test, the approach was found to be a convenient alternative to the conventional approaches.



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INTRODUCTION

It is known that air blast with enough intensity and duration can lead to contusion of gas-containing organs of an exposed test animal (1, 2). The gastrointestinal tract is particularly vulnerable to this type of injury. Field tests on sheep have shown that contusion marks usually appear on the large intestine and the stomach (3). Since these are the places where gas bubbles are most likely to accumulate, it is postulated that the wall deformation at the bubble site leads to the local lesion. However, direct connection between the injury site and the presence of gas bubbles was lacking. Furthermore, since the correlation between the dynamic loading parameters and the bubble geometry can only be established from direct observation and/or quantitative measurements, isolated GI tract tests became the logical approach for collecting such information.

Unperfused GI tract sections have two major drawbacks. Since an isolated unperfused GI tract has no blood pressure, there is little indication of the occurrence of injury. Because autolysis tends to take place shortly after the blood supply is cut off, little time is available for in-depth study and the test results are of questionable merit.

On the other hand, conventional isolated organ perfusion techniques, usually require a complex blood supply system, including a pulsating pump, oxygenator, foam suppression equipment, and provisions for prevention of blood cell damage, etc. (4). Furthermore, the blood volume of the donor animal alone is not sufficient to fill the extra capacity of the blood supply system and additional pooled homologous blood is necessary. This usually means sacrificing additional animals or appropriate dilution of the animal's blood, thus diluting the injury indicator.

One way to alleviate these drawbacks is to use the autologous perfusion technique. Despite its long length and complicated appearance, the blood supply of the rabbit GI tract initiates at only two major arteries: the cranial mesenteric artery and the caudal mesenteric artery. The former supplies the blood to the bulk of the GI tract extending from the duodenum to the descending colon while the latter covers the remaining length of the descending colon and the rectum. The venous blood flow from all of the tributaries return through the portal vein. This simple "inlet-outlet" configuration suggests the possibility of a simple surgical procedure to perfuse the isolated GI tract. By bisecting these blood vessels and extending them with silastic tubing, the test animal's GI tract would be perfused by its own cardiovascular system and the isolated organs could be tested in a separately controlled test chamber. The concept proved to be viable. The following is a detailed description of the procedure developed.

METHODS

The perfusion preparation procedure usually takes about 90 minutes. Exposing the GI tract to the room air during this preparation tends to cause it to dry out and alter its physical properties. A constant temperature recirculating saline bath, as shown in Figure 1, was therefore fabricated to serve as the surgical stage, to maintain the rabbit's body temperature (39° C) and keep the exposed GI tract moist.

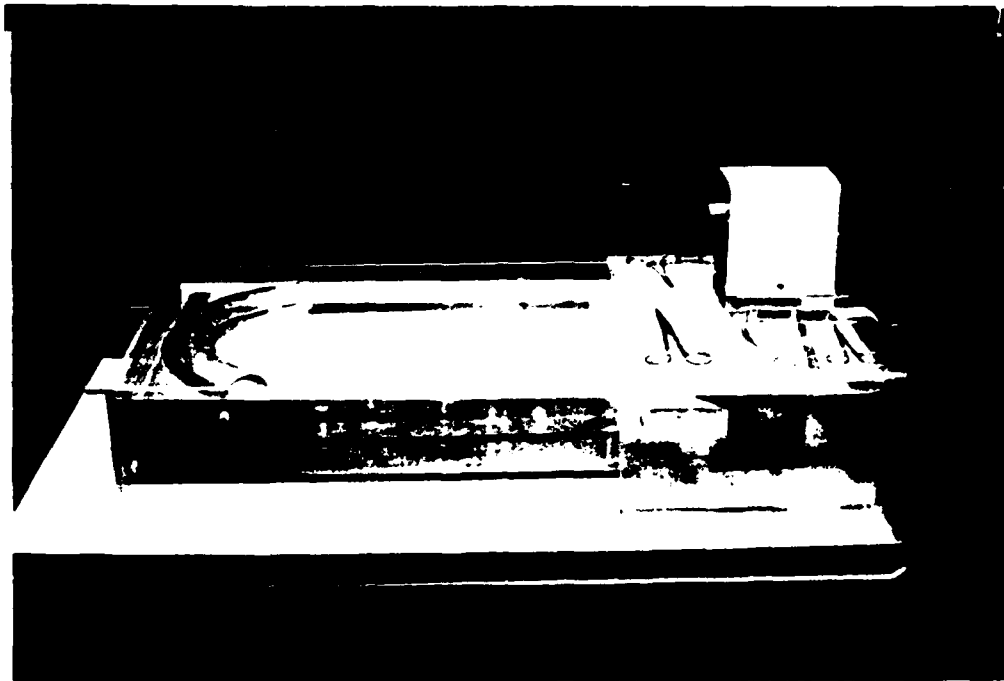


Figure 1. Constant temperature circulating saline bath for GI tract preparation.

Anesthesia for a typical 3 kg New Zealand white rabbit is accomplished by administering 2 cc ketamine (100 mg/ml) I.M. followed by I.M. injection of the combination of 1/2 cc xylazine (20 mg/ml) and 1/4 cc acepromazine (10 mg/ml). A waiting period of 30 minutes is usually required for the drug to take effect.

After anesthesia, the rabbit is restrained in supine position in the saline bath for surgical preparation. The general direct perfusion procedure involves the following steps:

1. Make a ventral incision caudally from sternum to pubis region and expose the viscera. Carefully lift the GI tract out of the abdominal cavity so as not to entangle or twist its blood vessels. Place it next to the animal on an elevated support in the saline bath. This reduces the unnecessary strain that might be incurred when the GI tract is lifted out of the abdominal cavity. Cover the GI tract with a damp cloth.
2. Locate the extreme caudal section of the descending colon. Ligate with two ligatures then sever the colon between them. Isolate the GI tract from the body by dissecting the connecting mesentery from the abdominal tissue until the caudal mesenteric artery is reached. Remove the fatty tissues to expose the caudal mesenteric artery. If caudal mesenteric artery is not to be perfused (refer to discussion below), then ligate it with two ligatures and dissect between them. Otherwise, prepare the caudal mesenteric artery for perfusion by removing the excess tissues attached to the blood vessel. Detailed preparation procedure is given in the caudal perfusion section below.
3. Dissect the connecting mesentery tissue along the back side of the abdominal cavity until the nodal junction of cranial mesenteric artery and portal vein is reached.
4. Locate, ligate, and sever the duodenum below the stomach. This frees the entire GI tract from the rabbit except for the connecting blood vessels.
5. Carefully clear away as much fatty tissue and fascia as possible to expose the cranial mesenteric artery and the portal vein. This step is critical for a successful catheterization of the portal vein, because the blood vessel will collapse when bisected and may be difficult to distinguish among the fatty tissue (and hence catheter placement).
6. Heparinize the rabbit I.V. in the ear to prevent blood clotting. Using 1/2 cc (10,000 USP/ml) has been found to keep the catheters patent for 3 to 4 hours.
7. Clamp off the cranial mesenteric artery and the portal vein with two hemostats. Make an incision in the portal vein and place a 14 gauge catheter caudally. Secure the catheter with a ligature. Slowly open the clamped mesenteric artery to check placement of the venous catheter. If blood flows freely, reclamp artery and cap venous catheter. If not, the catheter could have been misplaced in the mesentery or inserted too deep into the fatty tissue. In either case, retry the catheter placement until blood flows freely out of the catheter. Similarly, place a catheter in the cranial section of the portal vein and secure with a ligature. Connect the catheters with the prepared silastic tubing (0.078 inch diameter, filled with heparinized saline to displace any trapped air) to complete the venous loop. Bisect the ligated portal vein between the catheters to free the venous extension loop.

8. Repeat the procedure above for the cranial mesenteric artery by placing a 14 gauge catheter first in the caudal section then the cranial section. Connect the prepared silastic tubing between them. Bisect the cranial mesenteric artery between the ligated catheters to free the arterial extension loop.

The blood flow in the established direct perfusion loop is from the cranial mesenteric artery through the extension tubing into the GI tract and then through the second extension tubing return to the portal vein. Figure 2 shows the completed direct perfusion loop.

Caudal Mesenteric Artery Perfusion

The caudal mesenteric artery supplies blood to the lower section of the descending colon. Unlike the cranial mesenteric artery, it does not have an accompanying venous return; all venous flow goes through the portal vein back to the heart. Figure 3 shows the caudal mesenteric artery branch off for the abdominal aorta.

Direct perfusion of the caudal mesenteric artery is accomplished by inserting a pair of small catheters (22 gauge) at the dissecting joint and then connecting them to silastic tubing as described above for the cranial mesenteric artery. However, because of its small diameter (approx. 1 mm) and short length (1.3 cm), placement of the catheters was found to be quite difficult. Much practice is required to become proficient.

DISCUSSION

One of the limiting factors of the autologous perfusion technique is the vascular tubing geometry. Since the amount of resistance to the blood flow is directly proportional to the length/diameter ratio, small diameter and long length tubing will hinder the blood flow rate. Therefore, where feasible larger size and shorter length tubing is preferred.

Sodium fluorescein was used to validate the success of the perfusion procedure and define the cranial and caudal mesenteric circulation region. When the caudal mesenteric artery was not perfused, the fluorescein/saline mixture was not present in the circulatory region. Figure 3 is a picture taken under a UV light, and shows that there is a uniform glow over the bulk of the GI tract with a dark section left out of the perfusion loop indicating the effect of the nonperfused caudal section. The caudal region, however, is relatively short, extending approximately 5 cm upstream of the caudal mesenteric artery junction.

In view of the relatively small region affected, and the uncertainty involved as to whether an unobstructed blood supply could be maintained, it appears that the time-consuming surgical preparation of caudal mesenteric artery perfusion procedure may not be warranted for most experimental purposes. In this case, ligation of the descending colon 5 cm above the caudal mesenteric artery may be made to simplify the procedure and prevent the spread of autolysis of the unperfused section.



Figure 2. Complete intestinal loop.

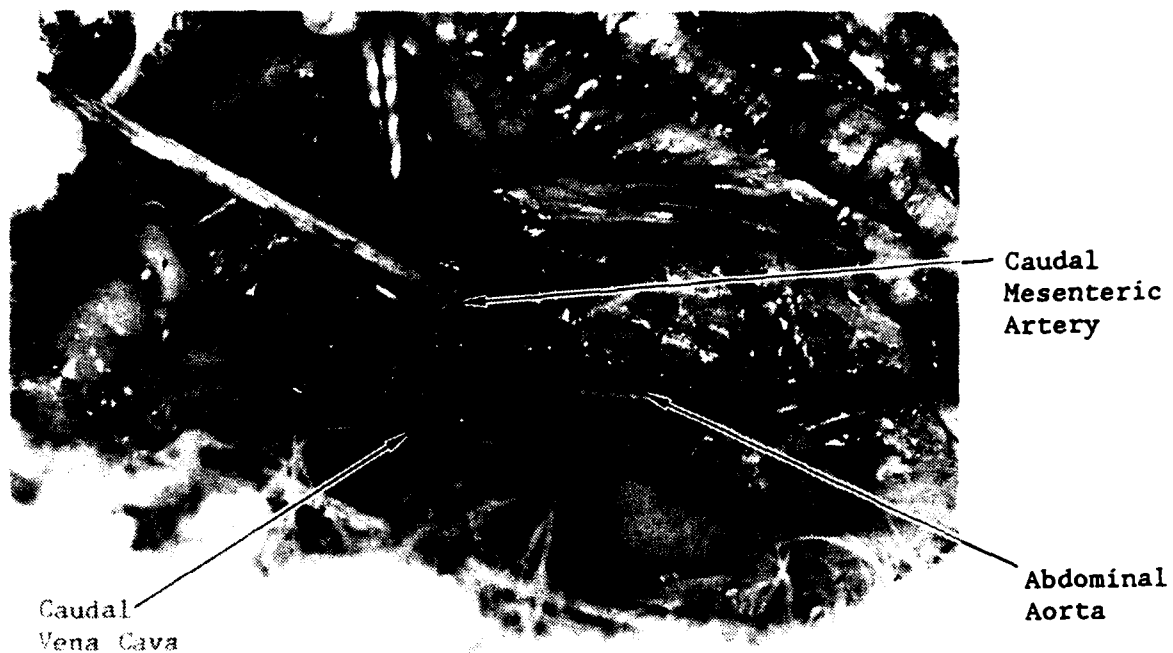


Figure 3. Caudal mesenteric artery/abdominal aorta junction.

The direct procedure involved for the placement of catheters at the cranial mesenteric artery and portal vein could prove to be quite demanding for an inexperienced operator because of the short length and tight working space at the junction. An alternative procedure to lighten the demand on the operator's dexterity is to use the abdominal aorta as the arterial blood supply and the caudal vena cava as the venous return. Since this bypass approach requires only one catheter placement at each of the junctions (the other bisected joint will be ligated), it could be used as a substitute for the standard procedure discussed above.

The bypass approach includes the following procedures. After the heparin injection (previous step 6), go to the following steps:

- a. Using blunt dissection, expose the abdominal aorta and the caudal vena cava just cranial to the caudal mesenteric artery.
- b. Ligate the abdominal aorta distally. Place a 14 gauge catheter cranially in the aorta and secure with two ligatures. Cap catheter.
- c. Ligate distal ligature on the caudal vena cava. Place a 14 gauge catheter cranially in the vena cava. Secure with ligatures. Cap catheter.
- d. Attach one end of a prepared perfusion tubing to the aorta catheter and the other end to the vena cava catheter. Open clamp to establish a temporary bypass loop and keep the catheters patent.
- e. Return to direct perfusion steps 7 and 8 as described earlier. Instead of inserting two catheters each to the cranial mesenteric artery and the portal vein, catheterize the artery caudally and the portal vein cranially. Ligate the other two remaining bisected sections.
- f. Return to the abdominal bypass loop. Clamp bypass loop. Disconnect the caudal vena cava joint and cap the vena cava catheter. Connect the bypass tubing to the cranial mesenteric artery catheter to complete the arterial flow. Quickly connect the free end of the portal vein perfusion tubing to the caudal vena cava catheter. Open both clamps. The perfusion loop is now completed.
- g. As shown in Figure 5, the circulation path of this approach is from the abdominal aorta to the cranial mesenteric artery through the GI tract out of the portal vein and returning to the heart through the caudal vena cava.

If care is taken to maintain body temperature and minimize blood loss during either the direct or bypass loop perfusion procedures, the anesthetized rabbit can maintain circulation in the perfused organ for several hours. Since this is a terminal experiment, the animal is usually euthanized in an accepted manner such as lethal injection.

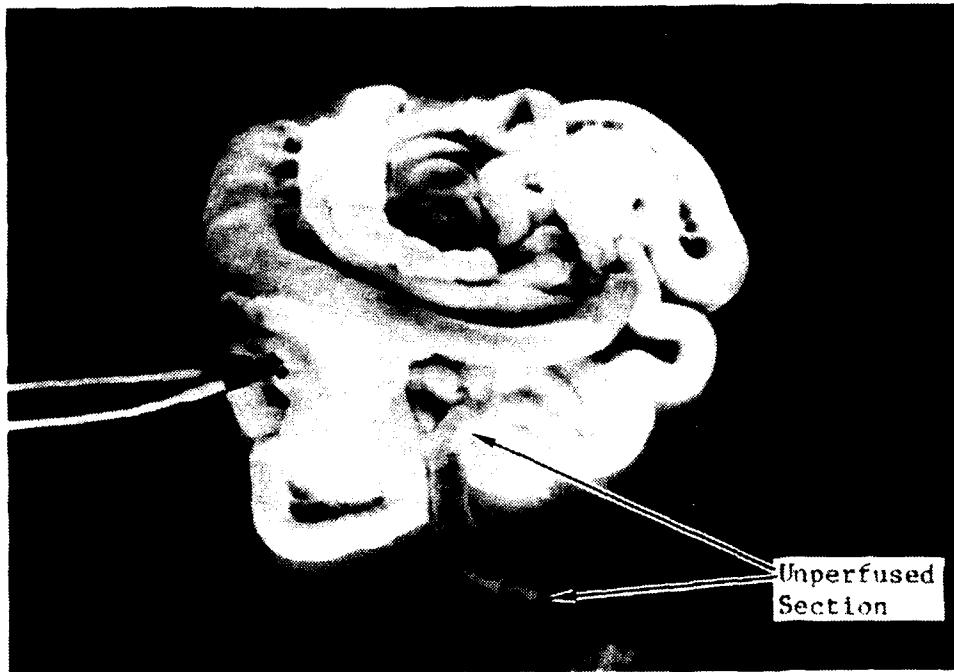


Figure 4. Region affected when caudal mesenteric artery is not perfused.



Caudal Abdominal
Vena Aorta
Cava

Figure 5. Perfusion loop of isolated GI tract. Flow is from abdominal aorta into cranial mesenteric artery, out of portal vein and into caudal vena cava.

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